Environmental controls on stable isotope ratios in New Zealand Podocarpaceae: implications for palaeoclimate reconstruction

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Abstract

Stable isotope ratios of various proxies are widely used for palaeoclimate reconstruction, and it is often assumed that isotope ratios reflect vegetation abundance or type. However, very little research exists on the isotopic equilibration of extant biomes under variable environmental conditions. In this study, carbon and oxygen isotope ratios from leaves of various Podocarpaceae genera, endemic to New Zealand, are linked to environmental parameters from the Land Environments New Zealand model. The dominant influence on stable isotope ratios within the majority of Podocarpaceae studied here is vapour pressure deficit (VPD). A simple latitudinal trend does not exist, and neither temperature nor rainfall (decoupled from VPD) are major controls on the stable isotope ratios. The results suggest that modern spatial heterogeneity in VPD affects the stable isotope values of vegetation, and that historic VPD variability would change the stable isotope ratios of Podocarpaceae without necessitating a change in vegetation type, density, or productivity. This represents an alternative model for temporal isotope change within geochemical proxies, and reinforces the

need for increased stable isotopic research in modern plant ecosystems to better understand modern, and eventually palaeoclimatic processes affecting the terrestrial biosphere.

Keywords:

Stable isotopes; New Zealand; Podocarps; leaves; environment; modern analogue; palaeoclimate

1. Introduction

Stable isotope ratios of various climate proxy archives are used extensively in palaeoclimate reconstructions (Brookman and Ambrose, 2013; Brown et al., 2013; Bussell and Pillans, 1997; Denniston et al., 1999; Dorale et al., 2010; Frappier et al., 2002; Frappier et al., 2007; Loader et al., 2013; McCarroll and Loader, 2004; Royer et al., 2013), however not all the processes affecting these ratios are understood. Past research has ascribed isotopic ratio variability in some terrestrial climate proxies (e.g., lake sediments, speleothems) to either changes in vegetation type (Denniston et al., 1999; Dorale et al., 2010) or biomass density (Baldini et al., 2005; Cosford et al., 2009). Isotopic discrimination in plant biomass, especially leaves, may affect climate proxy records. Stable-isotope ratios of plant material (especially $\delta^{13}C$) will ultimately affect soil isotopic ratios. Thus any proxy whose formation is partially dependent on soil processes will subsequently respond to changes in the terrestrial biosphere. To maximise the accuracy of palaeoclimate proxy record interpretations our understanding on how modern isotopic ratios of vegetation, regionally, respond to environmental change is critical (Fig. 1).

Podocarpaceae are coniferous C_3 trees regarded as the Southern Hemisphere equivalent of Pinaceae (Brodribb, 2011). A review focussing on the distribution and characteristics of Podocarpaceae was presented by Turner (2012; see references therein), but to date, very little isotopic research has been produced on Podocarpaceae.

Diefendorf et al. (2010) proposed that to better interpret $\delta^{13}C$ in palaeo-records, modern populations of similar species must be investigated under different environmental conditions

such as temperature, water availability and pCO_2. More accurate datasets would require a natural laboratory setting, and in this case New Zealand, which has a very varied climate, offers an excellent opportunity to produce such a dataset for Podocarpaceae, as an example of how isotopes may vary greatly within one family due to environmental conditions. This study measures $\delta^{13}C$ of bulk leaf material and relates that directly to environmental influences.

The stable carbon isotope ratio of leaf material, hereafter referred to as $\delta^{13}C_{leaf}$, is controlled by photosynthetic capacity (the rate of photosynthetic fixation of carbon) or stomatal conductance (the rate of gas exchange between the atmosphere and interstitial leaf space via the stomata). Differentiating between these two possibilities is achievable using the relationship between $\delta^{18}O$ and $\delta^{13}C$ of modern leaves (Scheidegger et al., 2000). In macrofossils, the $\delta^{18}O$ is a product of diagenesis and secondary alteration so it is not possible to use that relationship to establish stomatal conductance or photosynthetic capacity as the control for $\delta^{13}C$, except by comparing to a modern analogue. Once the relationship in modern leaves is known, it can be extended to leaf macrofossils of the same family to model the original $\delta^{18}O$ of that macrofossil. Therefore, the relationship between $\delta^{13}C$, $\delta^{18}O$, and environmental factors is critical for modelling macrofossil $\delta^{18}O$. Sampling $\delta^{18}O$ of fresh leaves allows interpretation of stomatal conductance and photosynthetic within each species or genus. According to Scheidegger's conceptual model (Fig. 2), the relationship of $\delta^{18}O$ to $\delta^{13}C$ indicates the dominance of stomatal conductance or photosynthetic capacity in $\delta^{13}C$ discrimination.

In this study, the isotopic variation of a number of species from the Podocarpaceae family across New Zealand were investigated. Podocarp species native to New Zealand are particularly useful because they are drought intolerant and their occurrence in the fossil

record have already been used to constrain climatic conditions: that climate is wet, mild and lacking in extremes (Coomes and Bellingham, 2011). Additionally, macrofossils from these species have been found in New Zealand and King George Island (Antarctica) (Fontes and Dutra, 2010; Turney et al., 1999), thus Podocarpaceae have the potential to provide regional palaeoclimatic information from the high-latitude Southern Hemisphere. Moreover, the study provides an example of how isotope ratios can vary across a plant family, which should be taken into account when studying other coniferous C_3 vegetation. We report that whole-leaf carbon isotope ratios within the same genus (Podocarpaceae) vary according to local climatic conditions, thus implying that they would change accordingly in the historic record due to a climatic shift.

2. Methodology

2.1. Field Area

Fieldwork was conducted in New Zealand, on both the North and South Islands. A large proportion of the land area of New Zealand is set aside as National Parks, allowing a huge area of native forest to flourish. With a wide variety of endemic and unique species, generally unpolluted watercourses, and a large latitudinal range producing a variety of environmental conditions, New Zealand is an ideal natural laboratory. Podocarpaceae do not grow in all regions found in New Zealand, because they are: (1) intolerant to hard frost and therefore do not grow at high altitude (with the exception of *P. alpinus* if conditions are correct); and (2) subject to xylem cavitation under drought conditions and hence are not found in semi-arid regions. *Nothofagus* dominates colder climate forest biomes (Leathwick, 2001) and native tussock grass dominates in semi-arid areas (Mark et al., 2003); furthermore, large-scale deforestation since the human settlement of New Zealand has limited the geographical extent of native forest, and not all Podocarpacae are found even where such native forests still exist.

Hence some areas of New Zealand have not been sampled and not all genera are sampled at every site.

All samples for this study were collected within a time window of 5 weeks during July and August of 2011. Sample types included leaves, leaflets, and water. After collection, leaf and water samples were regrettably not kept in constant conditions; samples were parcelled and sent by airmail to the UK in an uncontrolled environment and would have experienced changes in temperature and pressure. For example, *Phyllocladus* samples changed colour from green to brown during transit whilst all other specimens remained green.

Leaves or leaflets were taken from the family Podocarpaceae. The inclusion of the genus *Phyllocladus* in this family is debated (Keng, 1978; Molloy and Markham, 1999; Wagstaff, 2004). Leaflets were sampled at approximately 1.5 – 2 m above ground level and, where possible, from the side of the tree likely to experience the most sunshine (for example at the outside edge of a forested areas). Podocarpaceae are coniferous and do not drop their foliage seasonally, but produce new growth at the tip. To ensure a consistent sampling strategy, leaflets were taken from growth further down the branch, which grew approximately 2 - 3 years before. These stipulations were limited by the natural propensity of the trees to grow in dense rainforest and to grow very tall (up to 60 m) with the foliage only at the top few metres. This resulted in more sampling of juvenile trees which have morphologically different leaves from the adult tree in a number of genera. Reference samples were obtained from Dunedin Botanical Gardens where all genera in question were growing in the same conditions and at the same altitude.

To sample water, 10 cm^3 plastic vials were filled from lakes, rivers and streams at ~30 cm depth or base of water course, whichever was shallower. An air space of approximately 0.5 cm^3 was left at the top of each vial to allow for thermal expansion of the water whilst in transit. Sampled waters include glacial melt, shallow and deep lakes, rivers, streams, and water from hydrothermal areas.

2.2. Isotopic analysis

All analyses were performed in the Stable Isotope Biogeochemistry Laboratory, part of the Durham Geochemistry Centre at Durham University, UK. Whole-leaf $\delta^{13}C$ was determined using a Thermo-Finnigan Delta V Advantage isotope ratio mass spectrometer (IRMS) coupled with a Costech ECS 4010 Elemental Analyser. Samples of 0.3–0.5 mg were taken from a leaf or leaflet from each location, placed within a 6mm x 4mm tin foil capsule and sequentially dropped into a furnace where they were completely combusted. The resulting gases were passed through a gas chromatography column for separation and then measured in succession by the TCD detector. Isotopic measurements were initially taken from a transect of a token sample for each species, in order to establish any significant variation in $\delta^{13}C$ within the leaf. Each species was sampled at the same point on the leaf and the same area of growth to minimise any error from the natural variation in carbon within a leaf and tree. Data accuracy is monitored through routine analyses of in-house standards, which are stringently calibrated against international standards (e.g., USGS 40, USGS 24, IAEA 600, IAEA CH6, UREA, SPAR): this provides a linear range in $\delta^{13}C$ between +2‰ and –47‰ VPDB. Analytical uncertainty for $\delta^{13}C$ is typically ±0.1‰ for replicate analyses of the international standards and typically <0.2‰ on replicate sample analysis.

153 Whole-leaf $\delta^{18}O$ was determined using a Thermo-Finnigan Delta V Advantage isotope ratio
154 mass spectrometer (IRMS) coupled with a Thermo-Finnigan High Temperature Conversion
155 Elemental Analyser (TC/EA). The method for preparing samples for the TC/EA was similar
156 to that for the EA; except the mass weighed out was between 0.5–0.6 mg and silver foil
157 capsules of 5mm x 3mm were used. The samples were introduced to the TC/EA where they
158 underwent pyrolysis to produce CO and H_2. These gases were separated using an isothermal
159 gas chromatography column and passed to the IRMS for measurement. Data accuracy was
160 monitored through routine analyses of international standards (e.g., IAEA 600, 601, 602): this
161 provided a linear range in $\delta^{18}O$ between +71.4‰ and –3.5‰ VSMOW. Analytical
162 uncertainty for $\delta^{18}O$ was typically ±0.1‰ for replicate analyses of the international standards
163 and typically <0.2‰ on replicate sample analysis.
164
165 Each water sample was prepared using a sterilized 0.45μm filter attached to a 5 cm^3 syringe.
166 Approximately 1 cm^3 of water was filtered into a glass vial and sealed using a septum cap.
167 Isotopic measurements of $\delta^{18}O$ and δD were obtained by laser spectroscopy using a Los
168 Gatos Research DLT-100 liquid water isotope analyser (Berman et al. 2013). A suite of Los
169 Gatos Research water isotope standards was used and the analytical uncertainties for $\delta^{18}O$
170 and δD were typically better than ±0.1‰ and ±0.3‰ respectively. Replicate analyses of the
171 samples were typically <0.15‰ for $\delta^{18}O$ and <0.3‰ for δD.
172

173 ## 2.3. Data Analysis

174 Latitude and longitude of each sampling locale were entered into the Land Environments
175 New Zealand (LENZ) model, which then output modelled mean parameters used for this
176 study: mean annual temperature (MAT), mean minimum daily temperature of the coldest
177 month (TMIN), mean annual solar radiation (MAS) monthly water balance (R2PET), and

178 vapour pressure deficit (VPD). For accuracy and modelling details refer to Leathwick *et al.*
179 (2003a,b).
180
181 Bowen and Revenaugh (2003), and Bowen and Wilkinson (2002) presented the Online
182 Isotopes in Precipitation Calculator (OIPC) based on data from the International Atomic
183 Energy Association and the World Meteorological Organization Global Network for Isotopes
184 in Precipitation. OIPC values have been calculated for all water sampling locations from this
185 study for comparison (see section 3.1).
186
187 Stable isotope data derived from leaves were organised by genus rather than species or family
188 for a number of reasons: *P. totara* and *P. hallii* display a high degree of hybridisation
189 between the two species (Leathwick, 2001; Leathwick and Whitehead, 2001), and *D.*
190 *cupressinum* and *D. dacrydioides* are the only examples of these genera native to New
191 Zealand. *P. ferruginea* is much more readily identifiable than *P. taxifolia* such that the former
192 is our representative for the genus *Prumnopitys*. Further mixture analysis highlighted the
193 significant difference between juvenile and mature *D. cupressinum* specimens; the mean and
194 standard deviations of $\delta^{13}C$ from mature *D. cupressinum* versus juvenile specimens were well
195 predicted by the numerical model. Juvenile and mature *D. cupressinum* have significantly
196 different mean $\delta^{13}C$ values, and due to the difference in morphologies between the juvenile
197 and mature stages, and the growing conditions favoured by Podocarpaceae in general, this
198 observation is discussed in detail in section 3.3.
199
200 The statistical analysis package PAST (Hammer et al., 2001) was used to compute normality
201 using the Shapiro-Wilk, Jarque-Bera and Chi^2 tests; the Shapiro-Wilk values were used in
202 conjunction with the latter two for confirmation. When datasets were normally distributed,

Pearson's regressions were used to link isotopes to environmental variables, and where normality was rejected, Spearman's rank correlation coefficient was used. In each case *r* values and *p* values were calculated with *p* values below 0.001 taken to be the limit for very significant correlation, and 0.01 for significant.

Variability in regional climates due to landforms and weather patterns is very important for the distribution of Podocarp forests (Leathwick, 2001; Leathwick and Whitehead, 2001; Turner, 2012). Contour maps were created for the different environmental parameters used and for the isotopic datasets generated in this study. By comparing these maps one can qualitatively assess how the different environmental factors influence the correlation between the maps. For example, the Tongariro National Park in the centre of the North Island and the Southern Alps dividing the South Island are topographical highs, which strongly influence weather patterns. The contour maps for the different environmental parameters and isotopic datasets were generated using the contouring program, Surfer 10 (Golden Software).

3. Results & Discussion

3.1. Oxygen isotopes in New Zealand water samples

$\delta^{18}O_{water}$ measurements displayed significant positive correlation with latitude (r^2 = 0.31, p = 2.5x10^{-4}) as well as significant negative correlation with elevation (r^2 = 0.38, p = 3.8x10^{-5}). Temperature is partially a function of latitude and elevation so regression analysis of $\delta^{18}O_{water}$ with mean annual temperature (mat) and the mean minimum temperature of the coldest month (tmin) data from LENZ was conducted. Strong and statistically very significant positive correlations exist with both: $\delta^{18}O_{water}$ against MAT had an r^2 of 0.56 (p = 9.26x10^{-8}) and $\delta^{18}O_{water}$ against tmin results in 0.67 (p = 6.03x10^{-10}). These values are consistent with Rayleigh fractionation theory (Bowen and Wilkinson, 2002) even though these samples were

from streams, rivers, and lakes instead of direct precipitation and isoscapes reflect the relationship between tmin and $\delta^{18}O_{water}$ (Fig. 3).

OIPC (Bowen and Wilkinson, 2002) uses latitudes, longitudes and elevation to model $\delta^{18}O$ precipitation, but this study sampled surface water so a degree of difference would be expected between theoretical precipitation values and observed surface water values. Although a strong correlation between OIPC and sampled $\delta^{18}O_{water}$ exists, it is not as strong as might be initially expected (r^2 = 0.49). Sampled water displays more extreme values than OIPC (Fig. 4) and the means and variances of these data are not statistically similar (f = $2.9x10^{-9}$ and t = 0.04). OIPC calculates a monthly average $\delta^{18}O$ of precipitation which should account for seasonality. Surface water end members are included in our data; glaciers and estuaries were sampled. Sampled water also has a greater deviation from the global meteoric water line than OIPC values mostly likely due to interaction of precipitation with groundwater, aquifer units, and evaporation from rivers and lakes. The discrepancy in $\delta^{18}O$ range between them may stem from the effect of elevation where sampled values have a much stronger and more statistically significant correlation with elevation than OIPC values.

3.2. Leaf Isotopes

Water and leaves were not always sampled at the same locations so it is difficult to get an overall estimate of how $\delta^{18}O_{water}$ affects $\delta^{18}O_{leaf}$, however for the sites at which both were sampled, there is no significant correlation (r^2 = 0.09, p = 0.4, n = 9). No significant correlation allows the application of Scheidegger's conceptual model (Scheidegger et al., 2000) but it is still useful as a secondary source of inference supporting our conclusions.

Podocarpaceae leaf $\delta^{13}C$ is positively correlated with monthly water balance (r^2 = 0.18, p = 1.46x10^{-6}, n = 120) and VPD (r^2 = 0.13, p = 6.37x10^{-5}, n = 120). Leathwick and Whitehead (2001) demonstrated that the optimum VPD and root zone water deficit vary for different species of Podocarpaceae in New Zealand, potentially explaining the observed weak (but very significant) correlations; mixing of species and great diversity of environments may also contribute. The statistical significance of the relationship between $\delta^{13}C$ and $\delta^{18}O$ within the overall leaf data (p = 0.07) permits the use of the model produced by Scheidegger et al. (2000), which indicates that stomatal conductance is a slightly stronger control than photosynthetic capacity on $\delta^{13}C$ of Podocarpaceae in New Zealand (Fig. 2). Analysis of each genus separately corroborates this finding with stronger and more significant trends (section 3.3).

If leaf samples are considered based on their locations on either the North or the South Islands, it becomes apparent that $\delta^{13}C$ and $\delta^{18}O$ leaves for North Island have no significant correlations to any environmental parameters considered here. Coomes and Bellingham (2011) noted that Podocarpaceae thrive on post-catastrophic landscapes (e.g. volcanic) because their superior ability to capture and retain nutrients from poor soils allows them to outcompete angiosperms that would usually dominate on richer soils. On North Island, Podocarpaceae are at greatest abundance on post-eruptive landscapes whereas on South Island, landslides and floods allow them to regenerate their population while they temporarily have the advantage over angiosperms. Geothermal activity is a major aspect of North Island hydrology and has a significant impact of $\delta^{18}O$ of source water (Clayton and Steiner, 1975) which is likely to dampen correlations with environmental influences. $\delta^{13}C$ data from South Island Podocarpaceae are very significantly correlated with VPD (r^2 = 0.27, p = 1.83 x10^{-5}, n = 63) and water balance (r^2 = 0.2, p = 2.78 x10^{-4}, n = 63). $\delta^{18}O$ also has weak but significant

277 correlation with water balance (r^2 = 0.15, p = 0.048, n = 51). Water balance is inversely
278 correlated with VPD because they are both a function of precipitation and temperature. $\delta^{18}O$
279 and $\delta^{13}C$ are strongly correlated (r^2 = 0.37, p = 0.007, n = 51), which is consistent with
280 Scheidegger's model (Scheidegger et al., 2000) suggesting a primary control of stomatal
281 conductance over photosynthetic activity.
282

283 ### 3.3. Segregated data by species or genera

284 Initial r and p values of the entire data set displayed very little correlation to the
285 environmental parameters and mixing models indicated that at least two distinct normal
286 distributions existed within the data set). *Podocarpus* and *D. cupressinum* have significant
287 positive correlations with VPD and significant negative correlations with monthly water
288 balance in both $\delta^{18}O$ and $\delta^{13}C$ (Table 1), indicating a primary control of stomatal
289 conductance on $\delta^{13}C$, consistent with Scheidegger's model when $\delta^{18}O$ was regressed against
290 $\delta^{13}C$.
291

292 *D. dacrydioides* and *D. cupressinum* both have distinctive dimorphism between juvenile and
293 adult specimens. Mixture analysis for $\delta^{13}C$ *D. dacrydioides* indicated two normal
294 distributions within the data set although the correlation with VPD is significant throughout
295 the species. Acceptable sampling density of *D. cupressinum* provided an opportunity to look
296 for isotopic differences between juvenile and mature leaves. Juvenile leaf $\delta^{13}C$ and $\delta^{18}O$
297 display significant correlations with water balance and vapour pressure deficit (Fig. 5)
298 whereas mature leaf isotopes are not significantly correlated with the environmental
299 parameters considered (Table 2), although the two groups had statistically similar means. For
300 *D. cupressinum*, a juvenile form could therefore be a better palaeoclimate indicator than a
301 mature form because it appears to be much more sensitive to environmental stresses.

P. ferruginea $\delta^{13}C$ correlates with elevation rather than VPD and the $\delta^{18}O$ data indicate influences from MAT, MAS, water balance and VPD, although the latter is the strongest and most significant. $\delta^{18}O$ *P. ferruginea* display a correlation of 0.47 (p = 0.007, n = 13) with $\delta^{13}C$ indicating again a consistent relationship between the two isotopes and a controlling influence of stomatal conductance within this family.

Increased sunshine hours should cause an increased photosynthetic rate, which theoretically would cause a more negative $\delta^{13}C$ ratio, but correlations with MAS are not evident in $\delta^{13}C$ for *Podocarpus*, *D. dacrydioides*, *D. cupressinum* and *P. ferruginea*. However, increased sunshine hours would also cause an increased VPD; using the LENZ data, VPD and MAS have a strong positive correlation (r^2 = 0.4, p = 2.25 x10^{-14}, n = 118). Increased VPD causes stomata to close to minimise evaporation from the leaves hence $\delta^{13}C$ becomes less negative due to decreased gas exchange resulting in Rubisco sequestering more ^{13}C than usual. Because these two effects are opposed, the correlation with MAS would be highly dampened. Coomes and Bellingham (2011) note that Podocarpaceae are shade tolerant and reach light saturation at low thresholds compared with other C3 plants. *D. cupressinum* and *D. dacrydioides* exhibit dimorphism with pendant adult leaves, reducing surface area of exposed leaves exposed to direct sunlight and increasing the sunshine allowed into canopy areas; such morphological adaptations would also decrease correlations with MAS.

Phyllocladus differs in response to most parameters compared to the other genera/species studied here. $\delta^{13}C$ has a negative correlation with MAS but no other significant correlations and $\delta^{18}O$ had no significant correlations with climatic parameters (p > 0.1). Unlike the other plants, *Phyllocladus* $\delta^{18}O$ is negatively correlated to $\delta^{13}C$ although the correlation is not

significant (r^2 = 0.21, p = 0.13, n = 12). If we reject the significance of $\delta^{18}O$ to $\delta^{13}C$, it would be reasonable to propose that neither photosynthetic capacity or stomatal conductance have a significantly dominant influence on $\delta^{13}C$, however if significance is accepted, Scheidegger's model would propose that photosynthetic capacity is the main controlling factor on $\delta^{13}C$ which would agree with the correlation with MAS (Fig. 6). Turney et al (1999) carried out a similar study on *phyllocladus alpinus*, proposing VPD to be the main control on $\delta^{13}C$, but the *Phyllocladus* results presented here are inconsistent with that hypothesis. 86% of Turney's samples were from South Island whereas the majority of *Phyllocladus* samples from this study were from North Island, and geological differences between the islands may affect the results. *Phyllocladus* is disputed as a member of the Podocarpaceae family and is morphologically different from the other genera studied here because it has flattened stems called cladodes rather than leaves; it is therefore unlikely that *Phyllocladus* would have identical stomatal characteristics to the other genera considered.

3.4. Potential for palaeoclimate reconstruction

Presence of New Zealand Podocarpaceae in the fossil record constrains the palaeoclimatic interpretation, because they currently thrive only in a relatively narrow climate band within New Zealand. For Podocarpaceae forests dominated by these species to exist, the climate must be wet and cool but without extreme cold, and periodic catastrophic events must occur to allow regeneration of these conifers (Coomes and Bellingham, 2011). To further constrain the vapour pressure deficit and water balance, $\delta^{13}C$ of macrofossils can be compared with the modern day findings presented here. If *Phyllocladus* and a *Podocarp* fossil species occur in the same sequence, information about both Mean Annual Sunshine hours and Vapour Pressure Deficit of the palaeoclimate at time of deposition can be obtained.

The lack of strong latitudinal trends within these data and strong correlations with Vapour Pressure Deficit suggest that topography-induced environmental conditions are more important than latitudinally-induced conditions for affecting the stable isotope ratios of leaves (Fig. 7). This reinforces the concept that palaeoclimate proxies are best interpreted considering studies on stable isotopes in local vegetation.

4. Conclusions

The diversity of altitudes, latitudes, and types of environments from which the samples were obtained provides a robust test of the controls on stable isotope ratios of vegetation. *Podocarpus*, *D. cupressinum*, *D. dacrydioides,* and *P. ferruginea* $\delta^{13}C$ is primarily controlled by stomatal conductance responding to Vapour Pressure Deficit variability. The relationship between Mean Annual Sunshine hours and *Phyllocladus* $\delta^{13}C$ demonstrates that photosynthetic capacity is the dominant control. $\delta^{18}O$ ratios support these conclusions through statistical associations with Vapour Pressure Deficit and application of Scheidegger's conceptual model. These results are relevant for palaeoclimate reconstructions using a range of New Zealand proxies and macrofossils, and, when compared with $\delta^{13}C$ and $\delta^{18}O$ of other species worldwide, to construct more detailed global isotopic gradients. Additionally, this research suggests that local environmental conditions can directly affect climate proxy $\delta^{13}C$, suggesting that interpretations based on $\delta^{13}C$ proxies records should consider shifts in vegetation type, density, and climate (Fig. 1), because they can operate simultaneously and independently.

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6. References

Baldini, J.U.L. et al., 2005. Biomass effects on stalagmite growth and isotope ratios: A 20th century analogue from Wiltshire, England. Earth and Planetary Science Letters, 240(2).

Berman, Elena S F; Levin, Naomi E; Landais, Amaelle; Li, Shuning; Owano, Thomas., 2013. Measurement of δ18O, δ17O, and 17O-excess in Water by Off-Axis Integrated Cavity Output Spectroscopy and Isotope Ratio Mass Spectrometry. Analytical Chemistry, 85 (21), 10392-8

Bowen, G.J. and Revenaugh, J., 2003. Interpolating the isotopic composition of modern meteoric precipitation. Water Resources Research, 39(10).

Bowen, G.J. and Wilkinson, B., 2002. Spatial distribution of delta O-18 in meteoric precipitation. Geology, 30(4), 315-318.

Brodribb, T.J., 2011. A Functional Analysis of Podocarp Ecology. Smithsonian Contributions to Botany(95), 165-173.

Brookman, T.H. and Ambrose, S.H., 2013. Kangaroo tooth enamel oxygen and carbon isotope variation on a latitudinal transect in southern Australia: implications for palaeoenvironmental reconstruction. Oecologia, 171(2), 403-416.

Brown, A.G., Hatton, J., Selby, K.A., Leng, M.J. and Christie, N., 2013. Multi-proxy study of Holocene environmental change and human activity in the Central Apennine Mountains, Italy. Journal of Quaternary Science, 28(1), 71-82.

Bussell, M.R. and Pillans, B., 1997. Vegetational and climatic history during oxygen isotope stage 7 and early stage 6, Taranaki, New Zealand. Journal of the Royal Society of New Zealand, 27(4), 419-438.

Clayton, R.N. and Steiner, A., 1975. OXYGEN ISOTOPE STUDIES OF GEOTHERMAL SYSTEM AT WAIRAKEI NEW-ZEALAND. Geochimica Et Cosmochimica Acta, 39(8), 1179-1186.

Coomes, D.A. and Bellingham, P.J., 2011. Temperate and Tropical Podocarps: How Ecologically Alike Are They? Smithsonian Contributions to Botany(95), 119-140.

Cosford, J., Qing, H., Mattey, D., Eglington, B. and Zhang, M., 2009. Climatic and local effects on stalagmite delta C-13 values at Lianhua Cave, China. Palaeogeography Palaeoclimatology Palaeoecology, 280(1-2).

Denniston, R.F. et al., 1999. Speleothem evidence for Holocene fluctuations of the prairie-forest ecotone, north-central USA. Holocene, 9(6).

Dorale, J.A. et al., 2010. Isotopic evidence for Younger Dryas aridity in the North American midcontinent. Geology, 38(6).

Fontes, D. and Dutra, T.L., 2010. PALEOGENE IMBRICATE-LEAVED PODOCARPS FROM KING GEORGE ISLAND (ANTARCTICA): ASSESSING THE GEOLOGICAL CONTEXT AND BOTANICAL AFFINITIES. Revista Brasileira De Paleontologia, 13(3), 189-204.

Frappier, A., Sahagian, D., Gonzalez, L.A. and Carpenter, S.J., 2002. El Nino events recorded by stalagmite carbon isotopes. Science, 298(5593), 565-565.

Frappier, A.B., Sahagian, D., Carpenter, S.J., Gonzalez, L.A. and Frappier, B.R., 2007. Stalagmite stable isotope record of recent tropical cyclone events. Geology, 35(2), 111-114.

Hammer, Ø., Harper, D. and Ryan, P., 2001. PAST: Paleontological Statistics software package for education and data analysis. Palaeontologia Electronica.

Keng, H., 1978. GENUS PHYLLOCLADUS (PHYLLOCLADACEAE). Journal of the Arnold Arboretum, 59(3), 249-273.

Leathwick, J. et al., 2003a. LENZ Technical Guide. Ministry for the Environment, Wellington, pp. 237 pp.
Leathwick, J. et al., 2003b. Land Environments of New Zealand - Nga Taiao o Aotearoa. David Bateman Ltd, Auckland, pp. 184.
Leathwick, J.R., 2001. New Zealand's potential forest pattern as predicted from current species-environment relationships. New Zealand Journal of Botany, 39(3), 447-464.
Leathwick, J.R. and Whitehead, D., 2001. Soil and atmospheric water deficits and the distribution of New Zealand's indigenous tree species. Functional Ecology, 15(2), 233-242.
Loader, N.J., Young, G.H.F., Grudd, H. and McCarroll, D., 2013. Stable carbon isotopes from Tornetrask, northern Sweden provide a millennial length reconstruction of summer sunshine and its relationship to Arctic circulation. Quaternary Science Reviews, 62, 97-113.
Mark, A. et al., 2003. Tussock grasslands and associated mountain lands. In: J. Darby, R.E. Fordyce, A. Mark, K. Probert and C. Townsend (Eds.), The natural history of southern New Zealand. University of Otago Press, pp. 191-235.
McCarroll, D. and Loader, N.J., 2004. Stable isotopes in tree rings. Quaternary Science Reviews, 23(7-8), 771-801.
Molloy, B.P.J. and Markham, K.R., 1999. A contribution to the taxonomy of Phyllocladus (Phyllocladaceae) from the distribution of key flavonoids. New Zealand Journal of Botany, 37(3), 375-382.
Royer, A. et al., 2013. What does the oxygen isotope composition of rodent teeth record? Earth and Planetary Science Letters, 361, 258-271.
Scheidegger, Y., Saurer, M., Bahn, M. and Siegwolf, R., 2000. Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. Oecologia, 125(3), 350-357.
Turner, B.L., 2012. Smithsonian Digital Repository: Ecology of the Podocarpaceae in Tropical Forests.
Turney, C.S.M., Barringer, J., Hunt, J.E. and McGlone, M.S., 1999. Estimating past leaf-to-air vapour pressure deficit from terrestrial plant delta C-13. Journal of Quaternary Science, 14(5), 437-442.
Wagstaff, S.J., 2004. Evolution and biogeography of the austral genus Phyllocladus (Podocarpaceae). Journal of Biogeography, 31(10), 1569-1577.

7. Captions

Fig. 1*: Simplified models for interpretation of $\delta^{13}C$ excursions. $\delta^{13}C$ becomes less negative in the direction of x-axes arrows. Model A: A shift from sparse to dense vegetation causes $\delta^{13}C$ to decrease (e.g., Baldini et al. 2005). Model B: A shift of vegetation type from forest to prairie will cause $\delta^{13}C$ to increase (e.g., Denniston et al., 1999). Model C: Model tested by this study where a change in climatic conditions such as vapour pressure deficit or sunshine hours can cause $\delta^{13}C$ shifts within a stable biosphere.*

Fig. 2: *For scenarios a-h, $\delta^{13}C$ is plotted against $\delta^{18}O$ and related to stomatal conductance(g_i) and photosynthetic capacity (A_{max}) in a number of steps. Firstly the change in $\delta^{18}O$ is related to a change in vapour pressure deficit since $\delta^{18}O$ is controlled by stomatal conductance and stomatal conductance is primarily a function of relative humidity or in this study, vapour pressure deficit. Then $\delta^{13}C$ is linked to intecellular CO_2 concentration (c_i). In the 3rd step two possible causes for the change in ci are proposed in terms of changes in A_{max} and g_i, and then the more likely one chosen by considering vapour pressure deficit. This results in an output model of relative dominance of g_i or A_{max}. Adapted from Scheidegger et al. (2000).*

Fig.3*: Left: mean minimum temperature of the coldest month (LENZ) with sampling locations; green = leaves, blue = water, scale in °C. Right: $\delta^{18}O$ of sampled water with sampling locations..*

Fig. 4*: Data relative to Global Meteoric Water Line (green). OIPC $\delta^{18}O$ precipitation (blue) and $\delta^{18}O$ NZ water samples (red).*

Fig. 5*: Juvenile and mature D. cupressinum isoscapes. Colour scale is isotope ratio in ‰.*

Fig. 6: *Phyllocladus was sampled primarily from North Island and is correlated with Mean Annual Sunshine hours.*

Fig. 7*: Isoscapes a) Podocarpus leaf $\delta^{18}O$ b) Podocarpus leaf $\delta^{13}C$ c) Vapour Pressure Deficit d) Water Balance. Topographically controlled conditions provide a greater influence on leaf stable isotopes than latitudinal variations.*

Table 1*: Statistics for segregated data.*

Table 2: *Statistics for juvenile and mature D. cupressinum*

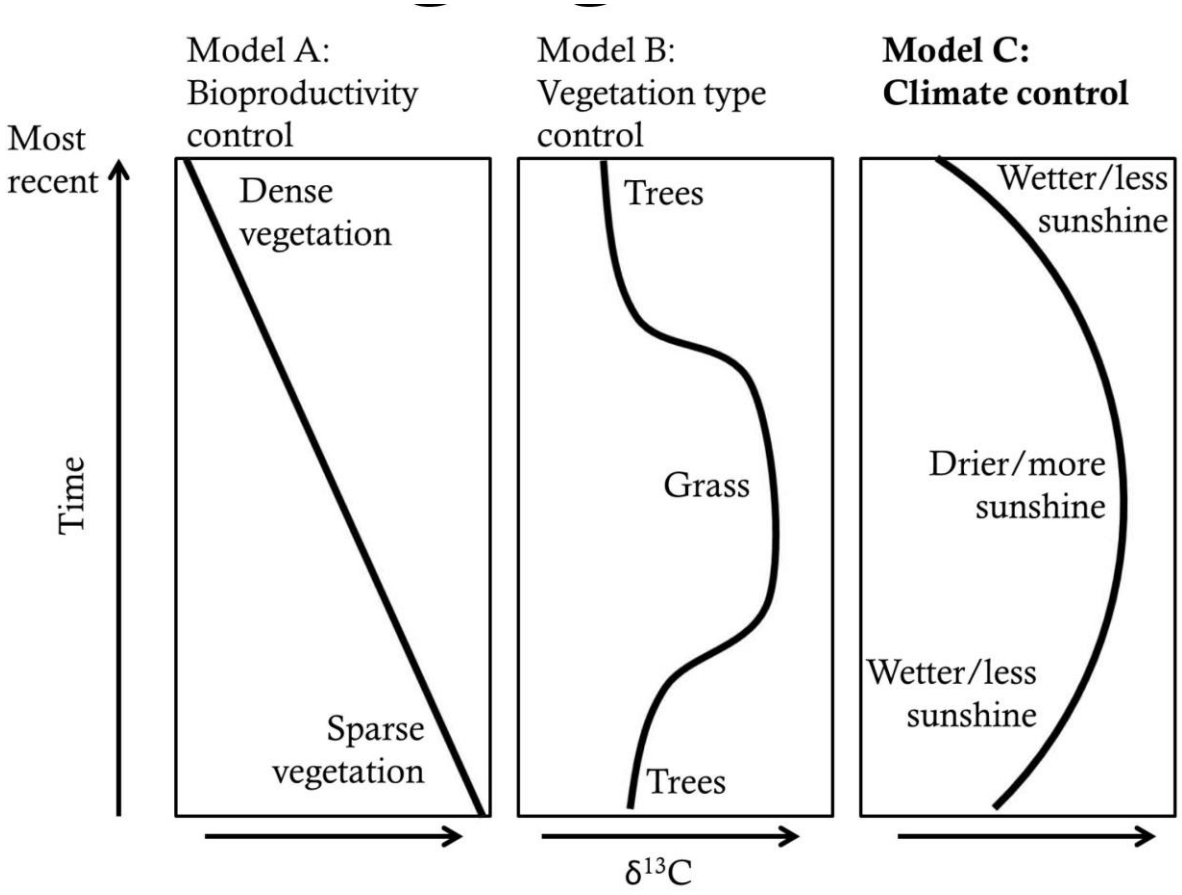
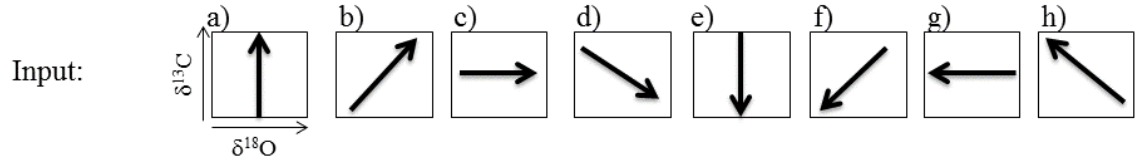

Model A:
Bioproductivity
control
Model B:
Vegetation type
control
Model C:
Climate control
Most
recent
Time
Dense
vegetation
Sparse
vegetation
Trees
Grass
Trees
Wetter/less
sunshine
Drier/more
sunshine
Wetter/less
sunshine
δ13C

Figure 1



$\delta^{18}O$	≈		↑		↑		↑		≈		↓		↓		↓	
VPD	≈		↑		↑		↑		≈		↓		↓		↓	
$\delta^{13}C$	↑		↑		≈		↓		↓		↓		≈		↑	
c_i	↓		↓		≈		↑		↑		↑		≈		↓	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
A_{max}	↑	≈	↑	≈	↑	↓	≈	↓	≈	↓	≈	↓	↑	↓	↑	≈
g_i	≈	↓	≈	↓	↑	↓	↑	≈	↑	≈	↑	≈	↑	↓	≈	↓
Selection based on VPD change	x			x		x		x		x	x		x		x	

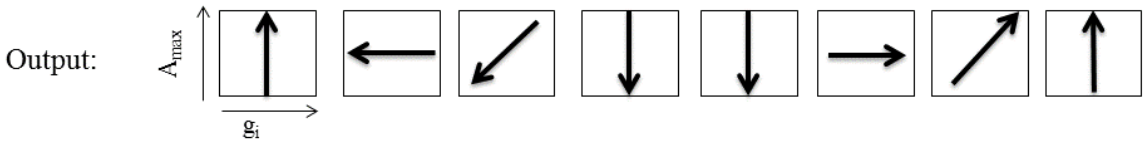
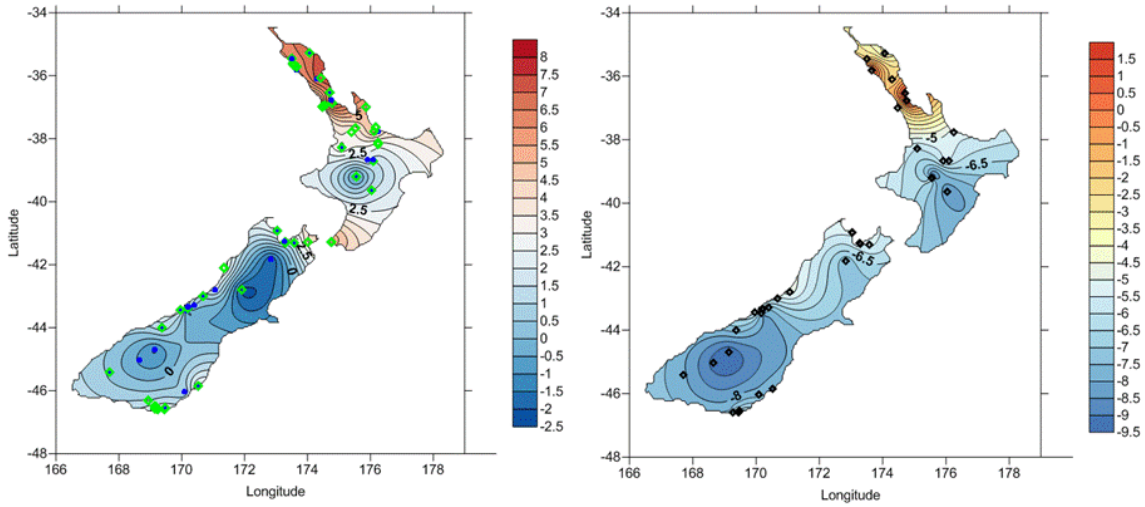


Figure 2


Latitude
Longitude
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-36
-38
-40
-42
-44
-46
-48
166
168
170
172
174
176
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8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
2.5
-5
-6.5
1.5
-3
-3.5
-4
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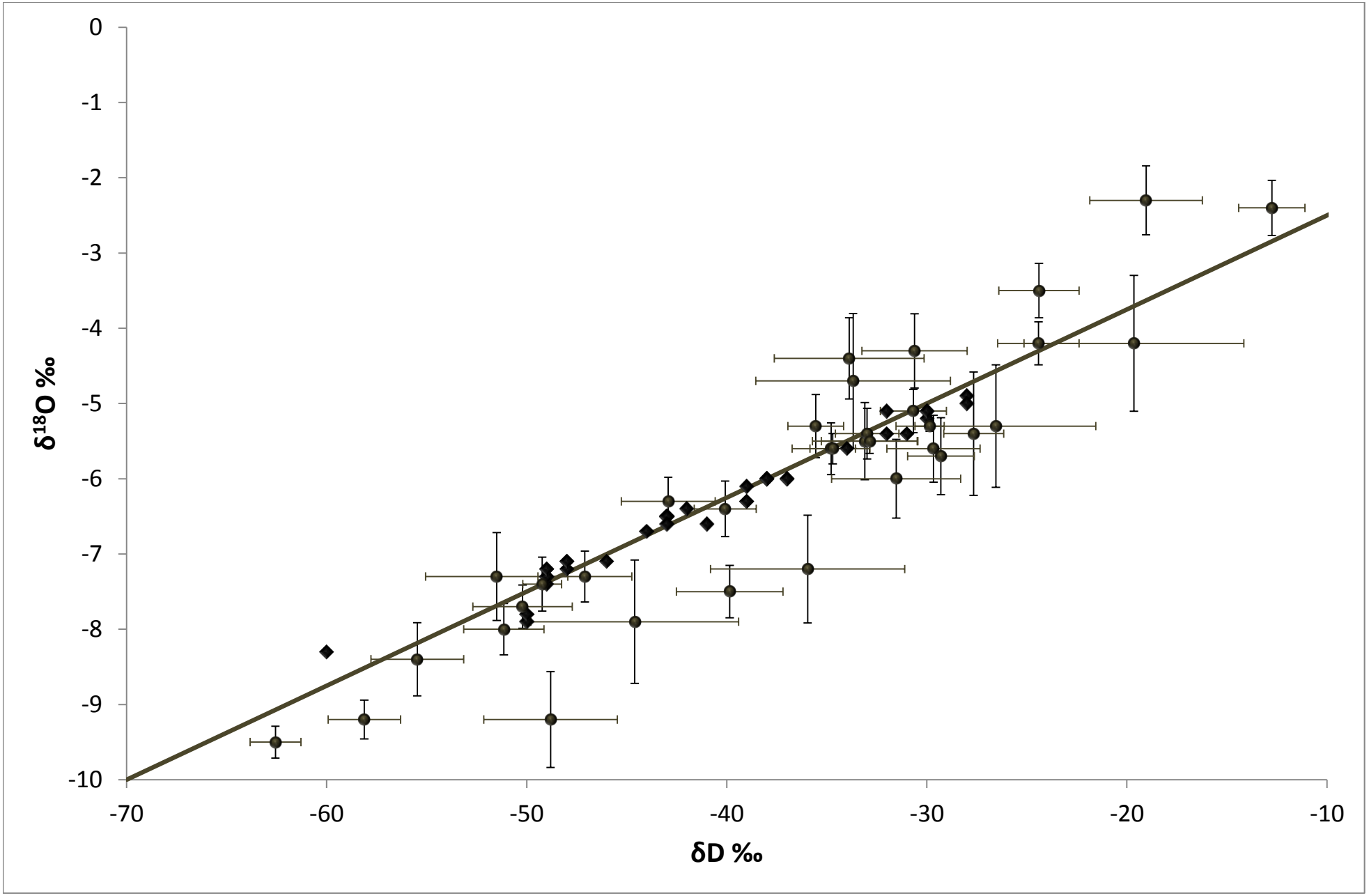
-5.5
-6
-7
-7.5
-8
-8.5
-9
-9.5

Figure 3


0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
δ18O ‰
-70
-60
-50
-40
-30
-20
-10
δD ‰

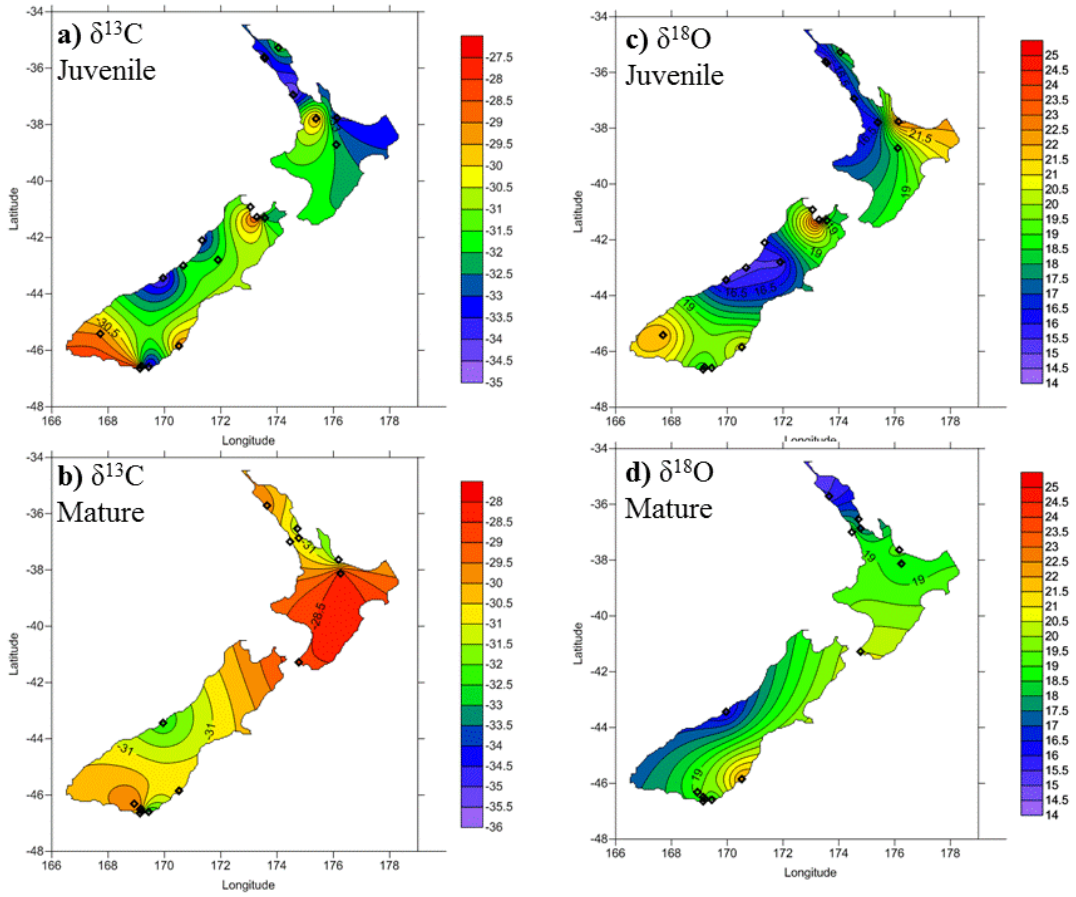
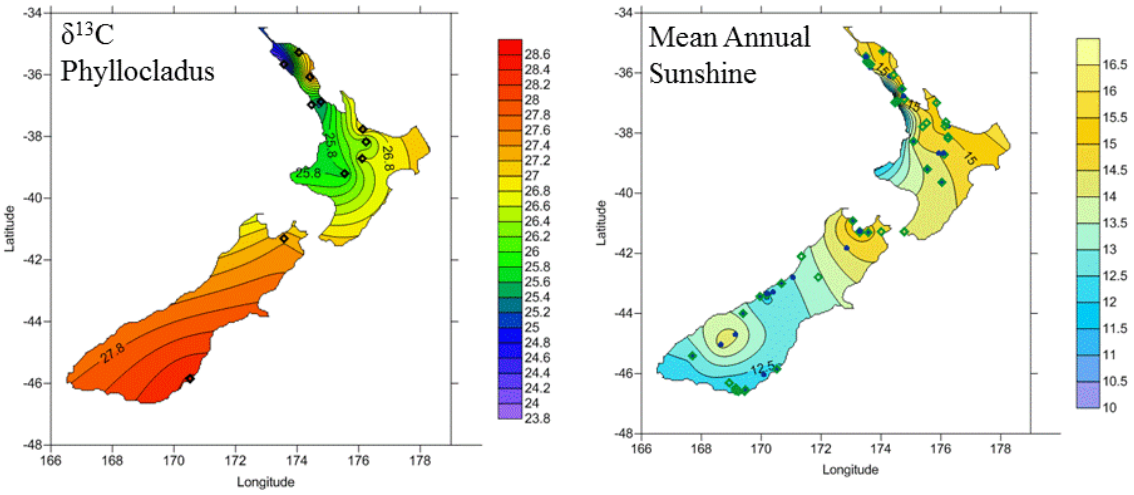

a) δ13C
Juvenile
b) δ13C
Mature
c) δ18O
Juvenile
d) δ18O
Mature
Latitude
Longitude

Figure 5

559



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561 Figure 6

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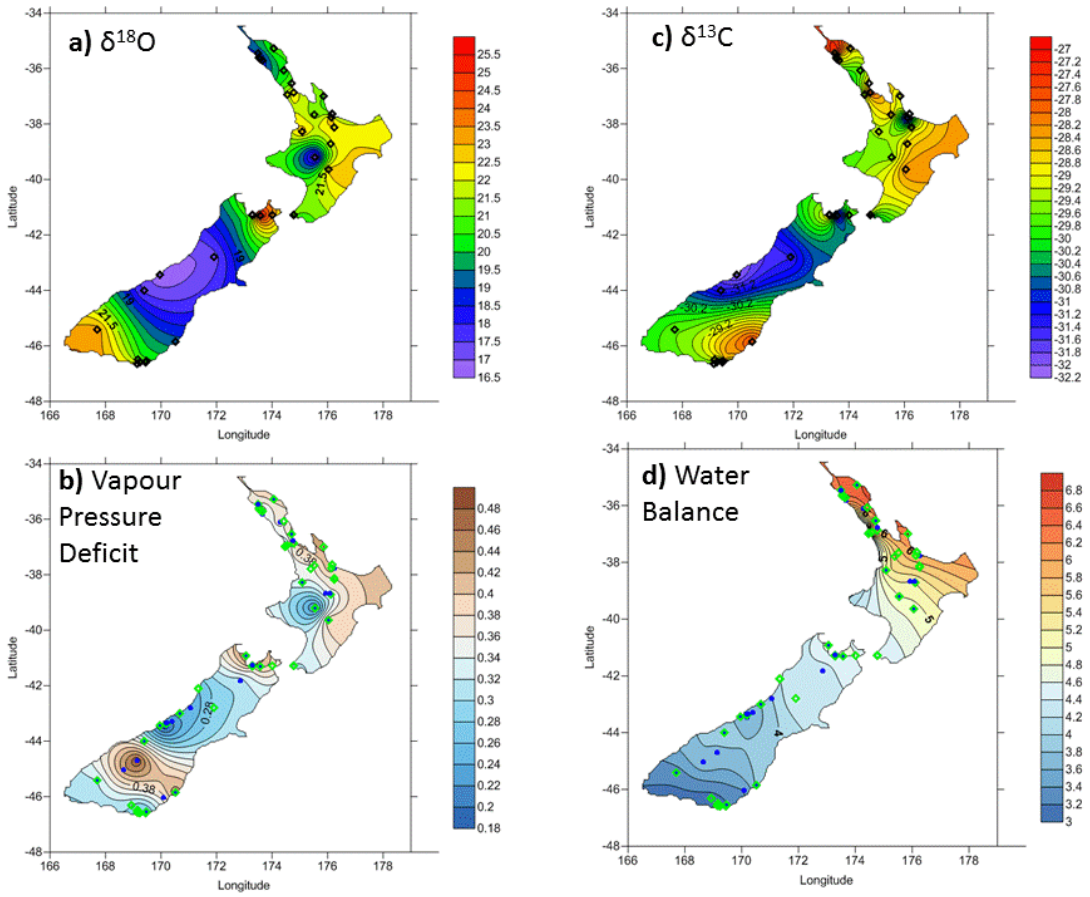
a) δ18O
b) Vapour Pressure Deficit
c) δ13C
d) Water Balance
Latitude
Longitude

Figure 7

Data set	Isotope	Parameter	Direction of correlation	Strength of correlation	p value (significance)	n
juvenile *d. cupressinum*	$\delta^{13}C$	Water balance	negative	0.16	0.092	19
		VPD	positive	0.16	0.094	
	$\delta^{18}O$	Water balance	negative	0.38	7.81×10^{-3}	17
		VPD	positive	0.44	3.77×10^{-3}	
mature *d. cupressinum*	$\delta^{13}C$	no significant correlation				12
	$\delta^{18}O$	no significant correlation				10

Table 2: *Statistics for juvenile and mature d. cupressinum*

Data set	Isotope	Parameter	Direction of correlation	Strength of correlation	*p* value (significance)	*n*
Podocarpus	$\delta^{13}C$	Water balance	negative	0.47	$8.77x10^{-4}$	38
		VPD	positive	0.22	0.003	
	$\delta^{18}O$	Water balance	negative	0.40	$1.51x10^{-4}$	31
		MAS	positive	0.16	0.01	
		VPD	positive	0.26	$3.55x10^{-3}$	
d. cupressinum	$\delta^{13}C$	Water balance	negative	0.12	0.055	31
		VPD	positive	0.10	0.079	
	$\delta^{18}O$	Water balance	negative	0.33	$1.68\ x10^{-3}$	27
		VPD	positive	0.37	$7.01\ x10^{-4}$	
p.ferruginea	$\delta^{13}C$	Elevation	negative	0.26	0.074	14
	$\delta^{18}O$	MAT	positive	0.28	0.06	13
		MAS	positive	0.35	0.03	
		Water balance	negative	0.32	0.04	
		VPD	positive	0.70	$1.73\ x10^{-4}$	
d. dacrydioides	$\delta^{13}C$	Water balance	negative	0.13	0.162	16
		VPD	positive	0.25	0.048	
Phyllocladus	$\delta^{13}C$	MAS	negative	0.20	0.143	12

Table 1*: statistics for segregated data.*